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A CONTAGIOUS DISEASE OF SALMON POSSIBLY OF VIRUS ORIGIN

BY R. R. RUCKER, W. J. WHIPPLE, J. R. PARVIN
AND C. A. EVANS

FISHERY BULLETIN 76

UNITED STATES DEPARTMENT OF THE INTERIOR, Douglas McKay, *Secretary*
FISH AND WILDLIFE SERVICE, Albert M. Day, *Director*

ABSTRACT

A disease, possibly of virus origin, is described which affected blueback-salmon and kokanee fingerlings (*Oncorhynchus nerka*), but did not infect fingerlings of the chinook salmon (*O. tshawytscha*), silver salmon (*O. kisutch*), or cutthroat trout (*Salmo clarki clarki*). The fish first became infected when they were 1 to 1.5 inches long and about 3 months old. Diseased fish were lethargic. Hemorrhagic areas often appeared at the base of the fins and in the isthmus. The stomach was distended with a milky fluid; the intestine appeared reddened and contained a straw-colored, watery fluid often tinged with blood. The bile appeared normal although the gall bladder was distended. Hyperemic areas frequently were present in the air bladder, peritoneum, and fat. Spinal deformities developed among some of the fish that survived the early stages of the epidemic. Therapeutic measures consisting of sulfonamides, antibiotics, and external disinfectants failed to control the disease. Rigid sanitary measures limited spread of the infection in some cases.

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CONTENTS

	Page
Outbreak of disease at the Leavenworth station, 1951	35
Disease among blueback-salmon fingerlings	35
Appearance of diseased fish	36
Therapeutic tests	37
Experimental transmission	38
Disease among kokanee fingerlings	38
Appearance of diseased fish	39
Experimental transmission	39
Outbreak of disease at the Winthrop station, 1949–51	41
Blueback brood of 1950	41
Blueback brood of 1948	41
Blueback brood of 1949	43
Kokanee brood of 1949	43
Experimental transmission	43
Relation to epizootic at Leavenworth	43
Discussion	44
Summary	45
Literature cited	46

A CONTAGIOUS DISEASE OF SALMON, POSSIBLY OF VIRUS ORIGIN

By R. R. Rucker,[1] *Fishery Research Biologist*, W. J. Whipple[1] and J. R. Parvin,[1] *Fish Culturists*, and C. A. Evans,[2] *Microbiologist*

OUTBREAK OF DISEASE AT THE LEAVENWORTH STATION, 1951

An outbreak of disease in 1951 among two groups of salmon fingerlings at the U. S. Fish and Wildlife Service station, Leavenworth, Wash., caused extensive losses. One of the affected groups was the blueback, or sockeye, salmon (*Oncorhynchus nerka*), which spends the first 12 to 24 months of its life in lake waters, and most of the next 2 years in salt water. It then returns to fresh water to spawn when about 4 years old. The other group was the landlocked salmon, or kokanee (*O. nerka*). It is of the same species, but differs from the blueback in that it spends its entire life in fresh water.

Transmission experiments indicated that a filtrable agent, presumably a virus, was the cause of this disease. The appearance of the sick fish, the findings at autopsy, and certain other characteristics of this epizootic closely resembled a disease seen previously among kokanee and blueback-salmon fingerlings at the Winthrop, Wash., station. Diet is not discussed, as the diets employed were used successfully at other stations in rearing similar fish.

This disease appears to be a significant problem in practical hatchery management. In addition, it is of interest in the field of comparative pathology in view of the probability that the causative agent is a virus. A noteworthy feature seen in a small number of the infected fish, particularly those that survive for relatively long periods, is a peculiar distortion of body shape comparable to scoliosis and lordosis as manifested in certain human diseases.

Facilities for this study were furnished by the School of Fisheries, University of Washington. Technical assistance of J. M. Alexander and S. W. Watson is gratefully acknowledged.

DISEASE AMONG BLUEBACK-SALMON FINGERLINGS

A group of about 1,400,000 blueback-salmon fingerlings which started feeding at the Leavenworth station on January 5, 1951, had insignificant losses—fewer than 300 daily—for 2½ months. These fish occupied 102 troughs. On about March 20, fish in one of the troughs were observed to have a higher mortality than those in the other troughs. Mortality data for the next 10 days are not available, but it is known that by the end of March about 13,000 fish were left in this trough. They were now approximately 2 inches long and 3 months old. Table 1 shows the number of these that died each day, April 1 to 10. After this date the surviving fish were used for therapeutic studies, and practically all of them eventually died from the disease.

TABLE 1.—*Daily mortality in 13,000 blueback-salmon fingerlings, Leavenworth station, April 1–10, 1951*

Date	Mortality	Date	Mortality
Apr. 1	163	Apr. 8	761
2	185	9	654
3	296	10	900
4	225		
5	270	Total	4,350
6	379	Percentage	33.5
7	517		

A similarly severe mortality occurred in five more troughs. In the six troughs, with a total population in excess of 80,000 fish, the mortality rate during March, April, and the first 3 weeks of May was virtually 100 percent. In contrast, during this same period the mortality rate in the remaining 96 troughs that contained some 1,300,000 fish was approximately 1 percent a month.

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These healthy blueback salmon were placed in outside ponds for rearing during the early part of June. They continued in excellent condition throughout the summer and had a very low mortality.

The water temperatures increased during the summer as shown in table 2; but when they began to drop in the fall, the mortality rate again increased slightly among the blueback salmon and the disease reappeared. This was indicated by the mortality of 0.4 percent for the 11-day period ended October 5. This represented a mortality rate more than twice that of the preceding 2 weeks and more than four times the rate during August and the first part of September. There was a progressive increase in daily mortality during the last 9 days prior to planting the fish on October 5.

TABLE 2.—*Biweekly mortality in salmon fingerlings with average water temperature, Leavenworth station, 1951*

[Original number: 1,400,000 blueback salmon and 788,000 kokanees]

Period ended	Blueback mortality	Kokanee mortality	Average water temperature
	Percent	*Percent*	*° F.*
Feb. 12	0.8		46
26	.7		46
Mar. 12	.6		46
26	1.1		45
Apr. 9	.8		45
23	1.2	3.6	45
May 7	2.4	3.4	45
21	2.5	2.4	44
June 4	.6	5.4	44
18	.2	1.2	46
July 2	.1	10.6	49
16	.2	14.4	56
30	.2	2.7	59
Aug. 13	.1	1.6	59
27	1	1.5	59
Sept. 10	.1	.8	54
24	.2	.5	52
Oct. 5	.4		49
9		1.0	49
Total	11.4	39.6	

Note.—Mortality is based on the percentage of fish alive at the beginning of each biweekly period. Total mortality is therefore not equal to the sum of the biweekly percentage mortalities.
Oct. 5 and Oct. 9 indicate planting dates, not biweekly periods.

The disease among the blueback-salmon fingerlings at the Leavenworth station in 1951 was demonstrated by a number of techniques to be contagious. The 1,400,000 young bluebacks were held in 102 troughs arranged in pairs separated by aisles. The troughs were tended by two workers using alternate aisles. Thus, each worker cared for the fish in the troughs on both sides of alternate aisles as shown in table 3. The pattern in which the disease spread among six troughs cleaned by one worker suggested an infectious disease, probably transmitted by equipment used to clean the troughs. As soon as it was suspected that an infectious agent was present, all cleaning equipment was sterilized between use in individual troughs by dipping it in a disinfectant. A 1:2,000 solution of roccal was used. It is remarkable that the infection was limited to the six troughs, presumably as a result of this precaution.

TABLE 3.—*Course of the disease through 6 troughs of blueback fingerlings, Leavenworth station, 1951*

[Total of 102 troughs and 1,400,000 fingerlings]

Adjacent pairs of troughs	Worker tending troughs	Order of infection	Date trough was infected
Trough 181	No. 1	No disease	
Trough 182	No. 2	Sixth	May
Trough 183	No. 2	Fifth	Apr. 2
Trough 184	No. 1	No disease	
Trough 185	No. 1	do	
Trough 186	No. 2	Third	Apr. 1
Trough 187	No. 2	First	Mar. 2
Trough 188	No. 1	No disease	
Trough 189	No. 1	do	
Trough 190	No. 2	Fourth	Apr. 2
Trough 191	No. 2	Second	Apr. 1
Trough 192	No. 1	No disease	

Appearance of Diseased Fish

A definite diagnosis could not be made either from the appearance of the sick fish or from the findings at autopsy. The findings conformed to a general pattern that might also occur in fish with other diseases.

Diseased fish appeared lethargic and did not react to stimuli, such as moving an object over the trough or jarring the trough. Normal fish would dart about, but sick fish usually did not move. After a few days, the abdomen was slightly bulged. A few fish showed hemorrhagic areas in some of the fins, at the base of paired fins, or in the isthmus; and the gills were pale.

In autopsied fish, the spleen was often light in color; the stomach was distended with a milky fluid, giving the impression of pyloric constriction; the gall bladder was filled with green bile, which in fish is typical of anorexia; the intestine was full of a watery, straw-colored fluid; and the large intestine often was red and apparently inflamed. Fluid expressed from the anus was yel-

lowish and generally tinged with blood. There was some muscular necrosis in the anal region.

Scoliosis developed during May in about 0.1 percent of the original population in the six troughs of diseased fish, and some fish with retracted heads were observed (see fig. 3).

The rest of the blueback fingerlings exhibited no characteristic disease symptoms throughout the summer, but during the first week of September, when water temperatures became progressively colder, diseased fish were again seen. At this time the fish were about 4 inches long and 8 months old. Diseased fish showed a reddened large intestine and a watery, bloody, yellowish discharge from the anus. About half of these had areas of fungus growth, predominately at the base of the pectoral, pelvic, or anal fins. These were the sites where hemorrhagic areas were seen on diseased fish in the spring. Fungus generally indicates debilitation or the presence of necrotic tissue. In September approximately half of the diseased fish exhibited spinal deformities; of these 60 percent had retracted heads, 30 percent scoliosis, and 10 percent lordosis (see fig. 1). Many fish showed no external signs of the disease other than these abnormal body shapes. A number of fish with spinal deformities had a surface growth of fungus, but autopsy of a few fish with scoliosis revealed no internal abnormalities.

Therapeutic Tests

The routine hatchery treatments of 1-hour baths in solutions of 1:4,000 formalin or of 1:100,000 pyridylmercuric acetate failed to influence the mortality rate. Therefore, sulfonamides and antibiotics were used experimentally on the fish that showed evidence of the infection. (See figure 2.)

When the fish in a trough became diseased they were divided into several groups and transferred to other troughs. The therapeutic agents were added to the food. Sulfamerazine, sulfaguanidine, sulfadiazine, and sulfamethazine were each used at the rate of 10 grams to 100 pounds of fish a day. The antibiotics were also added to the food at levels for 100 pounds of fish as follows: Aureomycin 1.5 grams in experiment A and 5 grams in experiment D; terramycin 2.5 grams; chloromycetin 2.5 grams; streptomycin 10 grams; and penicillin 1 million units in experiment C and 10 million units in experiment D.

The cumulative mortalities, shown in figure 2, are expressed as the percentage of the total mortality for each group. There was some difference in the total numbers of fish in the different troughs. At the beginning of an experiment, the number of fish in each trough was necessarily an estimate, and actual counts were made as the fish died. In experiment C, only an estimate is available (5,000 fish per trough) since the experiment was terminated at an early date because the drugs apparently were ineffective, and it was desired to set up another experiment at a higher drug level.

None of the therapeutic agents tested seemed to be of any benefit; the mortalities continued. Practically all fish died in each experiment regardless of the therapeutic agent employed.

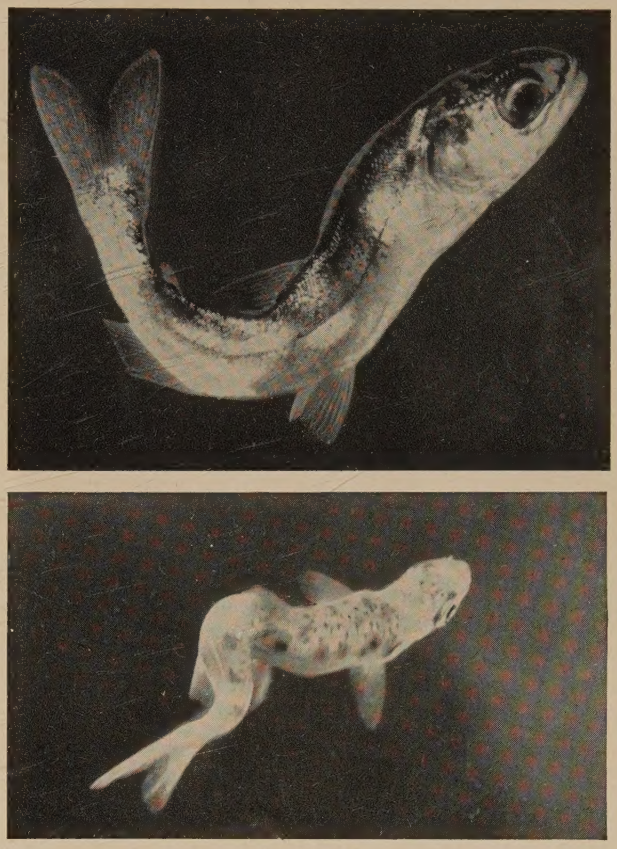

FIGURE 1.—Living blueback-salmon fingerlings from the Leavenworth station, showing lordosis in a 4-inch fish (upper) and scoliosis in a 3-inch fish (lower).

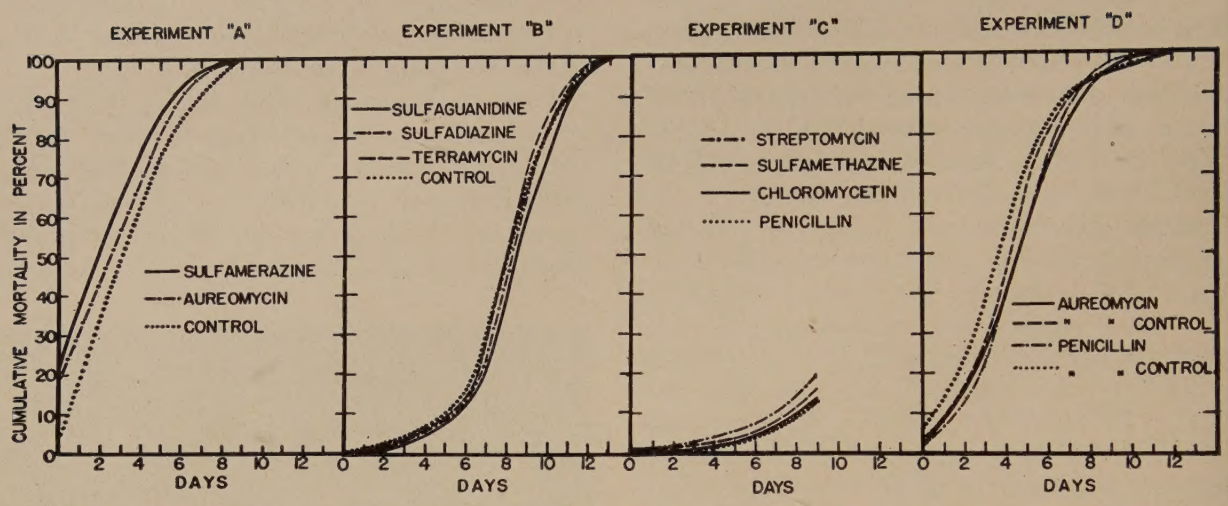

FIGURE 2.—Effect of sulfonamides and antibiotics on diseased blueback-salmon fingerlings at the Leavenworth station, 1951. The number of fish in each trial varied between 2,000 and 5,000 depending on the number of diseased fish available. Experiments A, B, C, and D were run at different times. Fish used in experiment C were transferred on the ninth day to experiment D. Practically all the fish died.

Experimental Transmission

To determine the nature of this disease, healthy blueback-salmon fingerlings were placed among diseased ones. They were obtained from a trough showing negligible mortality, and appeared to be in excellent health. One group of healthy fish was placed in a compartment with diseased fish; a second group was held in a separate compartment at the lower end of the same trough. Both groups showed signs of the disease after 12 days. Fish in the trough from which the healthy fish were taken remained well. Healthy fish placed in another trough of healthy fish, as a control, did not develop the disease. Silver-salmon fingerlings (*O. kisutch*) and cutthroat-trout fingerlings (*Salmo clarki clarki*) did not contract the disease when exposed in a similar manner to diseased fish.

No parasites could be demonstrated in diseased fish by the examination of stained tissue or by phase microscopy of fresh material. Similarly, attempts to isolate bacteria from diseased tissue yielded sterile cultures or organisms that were regarded as contaminants.

Broth and solid and semisolid media incubated at various temperatures and under both aerobic and anaerobic conditions were used. The composition of the media varied from rich media as brain-heart infusion (Difco), through peptones of various strengths, to dilute extracts of fish. Some media were enriched by the addition of ascitic fluid, yeast infusion, or fish extracts.

In two experiments, Seitz filtrates of suspensions of tissue from fish showing typical signs of the disease failed to infect a total of 20 inoculated fish. Control fish inoculated with unfiltered material died. However, bacteria capable of infecting injected fish were recovered from the controls. It is probable that the infecting virus was present, together with bacteria, in the unfiltered inoculum since in other similar experiments, when a coarser filter was used (7-pound-test Mandler filter), the filtrate was infectious and tests for bacteria were negative. The experiments are therefore not conclusive, but are interpreted as indicating that the infectious agent probably will not pass a Seitz filter.

DISEASE AMONG KOKANEE FINGERLINGS

While this disease was present among the blueback-salmon fingerlings, a group of 788,000 kokanee fingerlings also became affected at the Leavenworth station in 1951. As shown in table 2, the mortality amounted to about 40 percent of the population.

Evidence of the disease was first observed in one trough on May 18. The losses increased very rapidly during the next few days, and the fish in this trough were destroyed to prevent spread of the infection. By May 27, fish in another

trough showed signs of the disease. It appeared that the disease was kept somewhat under control after this time by sanitary measures and the fish were put in outside ponds on July 18. The disease became less prevalent in midsummer but increased in severity in the fall. It is possible that the decreased mortality in summer was related to the increase in water temperatures. Table 2 lists the water temperatures (range, 44° to 59° F.) for the period February 12 to October 9, and gives the biweekly mortality data. The daily mortality rate showed a definite increase during the latter part of the last 2-week period, although the table shows only the biweekly mortality. The characteristics of the diseased fish indicated that the disease was definitely approaching an epizootic stage when they were planted, having undergone a 39.6-percent mortality attributed to this disease.

Appearance of Diseased Fish

The kokanees were approximately 3 months old and about 1 inch long when the epidemic began in May. As a group, the larger fish appeared to suffer greater mortality than those of average or small size. Signs of the disease in the kokanees were similar to those in the bluebacks. The fish were apathetic; their gills were pale; some showed a flush of red at the base of the fins or on the operculum; and some exhibited popeye (exophthalmos). The spleen was often pale; hemorrhagic areas on the air bladder or necrotic areas in the musculature near the vent or dorsal area were found in some; and the gastrointestinal tract contained a watery fluid. The diseased fish appeared black from above while normal fingerlings were olive green.

At the end of the season spinal curvatures were not as frequent in the 2-inch kokanees as they were in the 4-inch bluebacks previously mentioned. About 0.1 percent of the total population of kokanees developed scoliosis, mainly during the period July to October. In the fall, when the mortality rate increased, fish with retracted heads became common. In figure 3, the characteristic posture is shown. The lateral view shows how the head was retracted. The dorsoventral distance was increased remarkably just posterior to the orbital area.

Experimental Transmission

The infectious nature of the disease among the kokanees was demonstrated in the following experiment. Twenty thousand kokanees from the last trough to show signs of the disease were divided equally between two adjacent troughs. One group served as the control. For a period of 2 weeks the other group was treated every other day for 1 hour with 1:100,000 pyridylmercuric acetate. There was no diminution of the mortality rate.

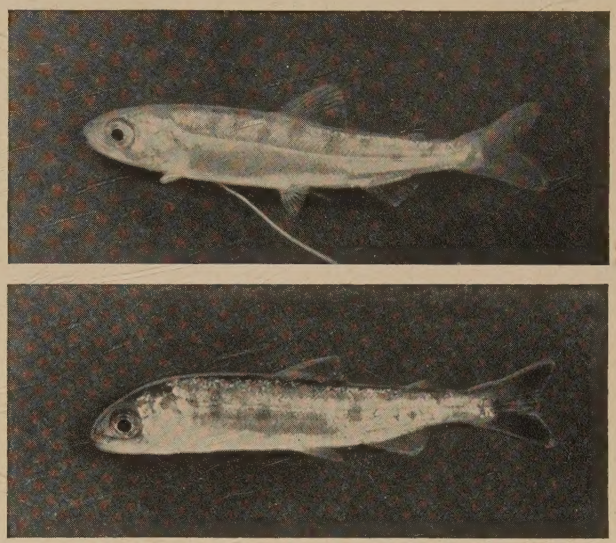

FIGURE 3.—Normal (upper) and diseased (lower) 2-inch living kokanee fingerlings from the Leavenworth station. The diseased fish shows the retracted head.

In another experiment, two adjacent troughs were each stocked with 10,000 healthy kokanees. One trough was cleaned with equipment also used on the troughs containing diseased kokanees, the other with separate equipment disinfected with roccal. About 12 days after initiation of the experiment an increased mortality was noted among the fish in the trough cleaned with contaminated equipment. During the next 48 hours, an additional large number of fish became sick, showing characteristics of the disease. The disease did not appear in the second group, and the experiment was terminated at this time. The infectious nature of the disease affecting the kokanees was thus shown to be similar to that affecting the blueback-salmon fingerlings.

As in the blueback salmon, a search for parasites that could be responsible for the disease was unsuccessful. Similarly bacteriological studies failed to provide an explanation for it. To test for a filtrable infectious agent, a suspension was prepared by blending one group of moribund kokanees with Ringer's solution; a second suspension was prepared from a group of five fish with scoliosis and five with lordosis by grinding them in Ringer's solution with mortar and pestle. Both suspensions were centrifuged.

A portion of each suspension was passed through a 7-pound-test Mandler filter that retained bacteria. Portions of both filtered and unfiltered material were heated at 80° C. for 15 minutes. Each of the four preparations was injected into a group of 20 2- to 3-inch healthy kokanee fingerlings. Ten of each group were injected intramuscularly with 0.1 milliliter and 10 intraperitoneally with the same amount.

Ninety-five percent of the fish injected with the unfiltered and unheated material from both original suspensions died within a week (table 4) with evidence of inflammation and necrosis of the muscle in the area of the injection or inflammation throughout the body cavity and viscera. Cultures from these fish indicated the presence of miscellaneous bacteria. Death was probably the result of infection by bacterial contaminants, and not related to the disease in the hatchery. No fish died among those injected with the heated material.

TABLE 4.—*Mortality of healthy kokanee fingerlings injected with fractions of diseased kokanees Leavenworth station, 1951*

[IP—group injected intraperitoneally; IM—group injected intramuscularly; each group comprised 10 fish]

Date	Deaths among fish injected Sept. 28, 1951, with suspension from moribund kokanees								Deaths among fish injected Sept. 30, 1951, with suspension from kokanees with scoliosis and lordosis							
	Unfiltered				Mandler filtrate				Unfiltered				Mandler filtrate			
	Unheated		Heated		Unheated		Heated		Unheated		Heated		Unheated		Heated	
	IP	IM	IP	IM	IP	IM	IP	IM	IP	IM	IP	IM	IP	IM	IP	IM
Sept. 30	1															
Oct. 1																
2	2	7														
3	4	2			1					2						
4	1					1			1	4						
5	1					1			3	2						
6	1								4	2						
7													3	3		
8					2	1										
9						2								2		
10													2	1		
11																
12						(1)										
13						1							1			
16									1							
23						1										
Total	10	9			3	7			9	10			6	6		
Combined mortality, Nov. 30 (percent)	95		0		50		0		95		0		60		0	

[1] One missing.

Fifty percent of the fish that were injected with the Mandler filtrate of the suspension of moribund fish died in a period of 1 month. They developed petechiae at the base of paired fins, on the isthmus, or on the side of the body. The viscera were inflamed, and often the inoculation site showed inflammation with some necrosis in the muscle. Cultures of kidney and muscle on agar yielded colonies of a variety of bacteria on about half the plates, presumably contaminants or secondary invaders. The other plates remained negative.

Sixty percent of the fish died that were injected with the Mandler filtrate of the suspension from kokanees affected with scoliosis and lordosis. They also showed areas of inflammation on the body surface, at the base of the fins, on the isthmus, on the viscera, fat, and especially on the intestine. Bacteria were not always recovered from the kid-

ney and muscle cultures from dead fish, but usually these cultures yielded miscellaneous forms, presumably contaminants or secondary invaders. Thirty-four days after inoculation, one intramuscularly inoculated fish showed a slight reddening in the dorsal-fin area and a tendency toward lordosis. Photographs of this fish demonstrate the progressive nature of the disease (fig. 4). One intraperitoneally inoculated fish showed the first signs of developing scoliosis 42 days after injection. This fish was photographed to show the progressive development of scoliosis (fig. 5). Observations on this experiment were terminated November 30, 1951, 5 weeks after the last mortality occurred.

OUTBREAK OF DISEASE AT THE WINTHROP STATION, 1949–51

BLUEBACK BROOD OF 1950

At the time of the outbreak of disease at the Leavenworth station in the spring of 1951, there was a similar occurrence of disease at the Winthrop station. Blueback-salmon eggs taken at Winthrop in the fall of 1950 were hatched both at the Winthrop and the Leavenworth stations. Fingerlings from the eggs retained at the Winthrop station were raised without significant loss up to the time of their release on April 20, 1951. A group of 69,000 fingerlings from the same group of eggs, hatched and raised at the Leavenworth station to a size of about 1½ inches, was transferred to the Winthrop station on March 8, 1951. Mortality among this group of fish was above normal in April, and most of the fish were lost from disease during May when they were about 2½ inches long and 5 months old. The disease began in one trough, approximately 7 days later was found in two more troughs, and then became general.

The disease was characterized by the rapidity with which it spread and by the death of many fish without apparent gross abnormalities. Many fish had hemorrhagic areas on or at the base of the fins and on the isthmus. Some had fungus growing on the peduncle, around the dorsal fin, or at the base of the pectoral fins. The gills were a normal red, but the spleen often appeared pink. Moribund fish showing external lesions had normal-appearing spleens. No solid material was found in the stomach which, in common with the intestine, contained a clear to yellowish fluid. There was no response to sulfadiazine or aureomycin therapy. Scoliosis developed in about 5 percent of the diseased fish.

BLUEBACK BROOD OF 1948

Losses among the blueback-salmon fingerlings and yearlings at the Winthrop station over the previous several years had on occasion been rather high. Necrosis of the kidneys was common and bacteriologic studies indicated that a short Gram-positive rod of the kind described by Earp (1950) was the etiologic agent. During the winter of 1949–50, sulfadiazine therapy was employed in an attempt to reduce the losses among the blueback-salmon fingerlings (Winthrop brood of 1948). Rucker, et al. (1951) working with a group of these fish found that sulfadiazine controlled the kidney infection among the population at the hatchery, but the mortality rate continued high—about 10 percent a month of 40,000 fish—during January, February, and March at which time the fish were released.

It is considered probable that the continuing mortality was due to the same agent encountered at the Leavenworth station in 1951. Two other fish pathogens were isolated from moribund fish of this lot of fish at the Winthrop station during 1949–50 and tentatively identified as *Pseudomonas hydrophila* and *Ps. fluorescens*. Both produced fatal infections when injected into healthy fingerlings. While both may occur in nature as fish pathogens, it was believed that they were not a primary cause of the prevalent disease because, after the kidney infection was controlled, they did not occur consistently in infected fish (see Schäperclaus 1941, Layman 1949).

Material passed through a Seitz filter was used in tests for a filtrable agent in this group of fish. No evidence of a virus infection was demonstrated. A Seitz filter retains some viruses that will pass through Mandler filters. It will be recalled that in one experiment a Seitz filtrate of extracts of diseased fish from the Leavenworth outbreak was also noninfectious; in another, the agent passed through a Mandler filter and was shown to be infectious.

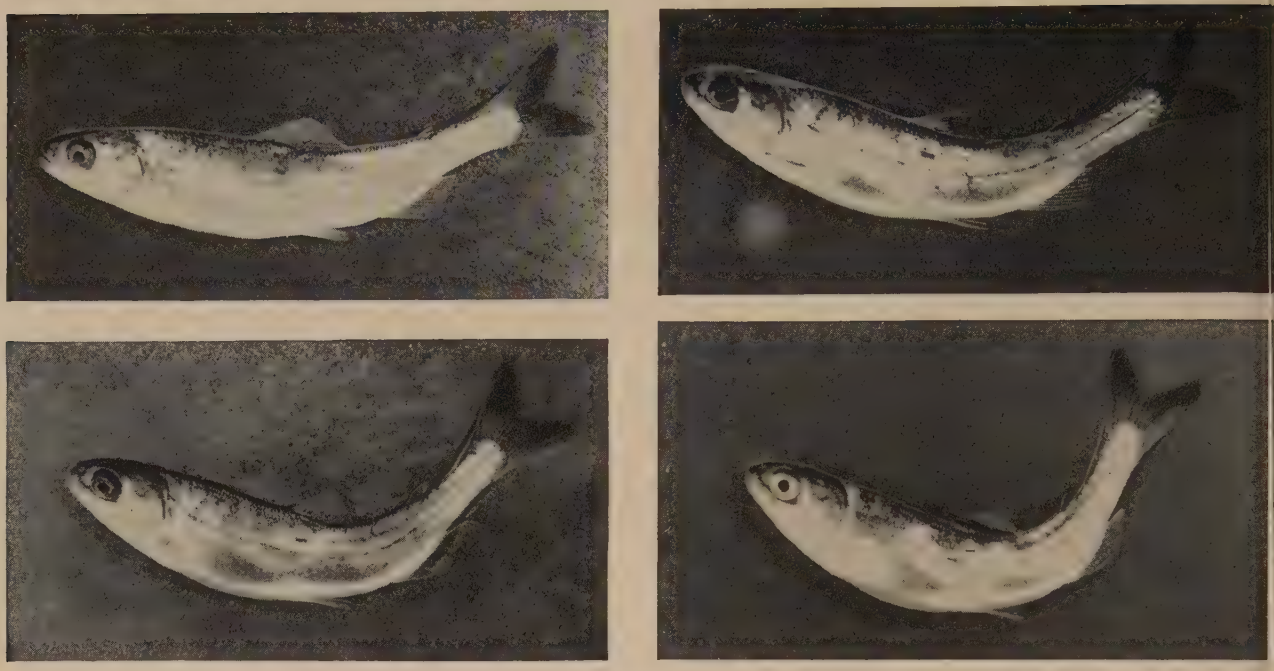

FIGURE 4.—Development of lordosis in a 3-inch kokanee injected intraperitoneally Sept. 30, 1951, with Mandler filtrate of suspension made from fish with lordosis and scoliosis. The first symptoms were observed on Nov. 3, and progression of the disease is shown in the photographs taken Nov. 9 (upper left), Nov. 16 (upper right), Nov. 23 (lower left), and Nov. 30 (lower right).

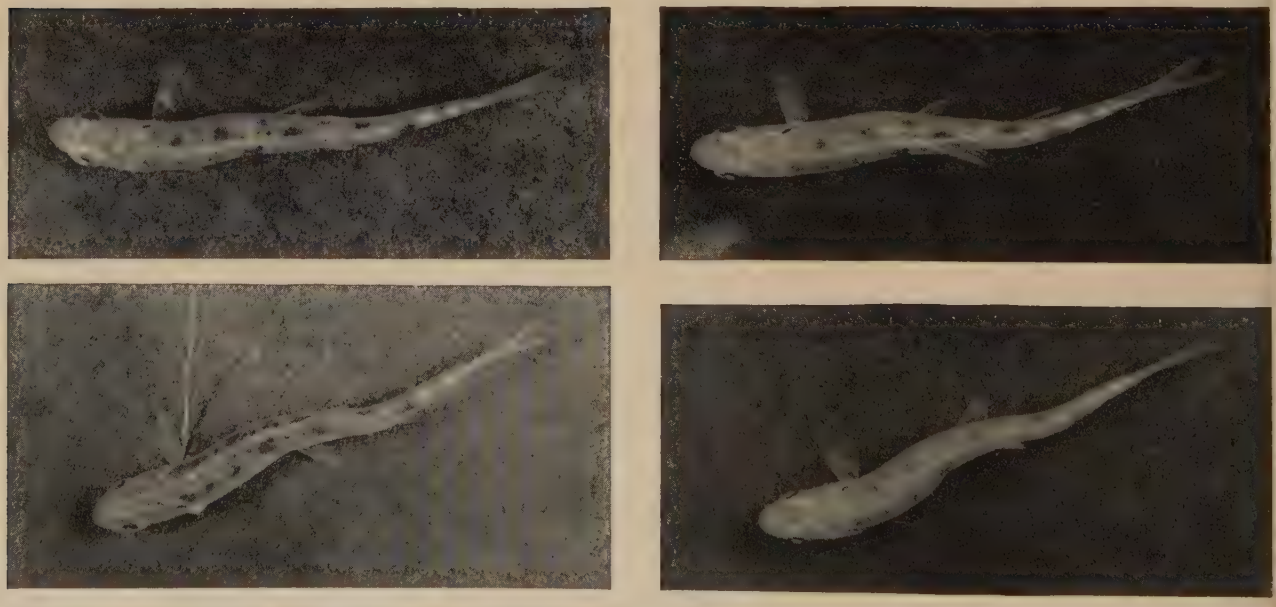

FIGURE 5.—Development of scoliosis in a 2-inch kokanee injected intraperitoneally Sept. 30, 1951, with Mandler filtrate of suspension made from fish with lordosis and scoliosis. The first symptoms were observed on Nov. 11, and progression of the disease is shown in the photographs taken Nov. 12 (upper left), Nov. 16 (upper right), Nov. 23 (lower left), and Nov. 30 (lower right).

BLUEBACK BROOD OF 1949

A group of about 140,000 small blueback-salmon fingerlings (Winthrop brood of 1949) showed a tremendous mortality at the Winthrop station during February 1950. These fish had hatched in January 1950, started feeding, and reached a length of about 1 inch in the month. The mortality occurred predominantly among the larger fish in the group.

The outbreak was characterized by a sudden increase in mortality. Most of the fish in an infected trough would die within a period of 2 weeks. The disease would appear in one trough, then show up in one or two others, gradually spreading from trough to trough, each suffering severe losses in its turn. Finally, the disease spread through the entire population. A loss of 80 percent in this group during the month of February was attributed to this disease.

Diseased fish were characterized by apathy, and they did not respond normally to moderate stimuli. A few fish developed scoliosis in March, and by May about 5 percent of the remaining stock of 2,000 fish showed this affliction. Scoliosis was not found among those that died during the earlier, acute stage of the epidemic.

At autopsy, small hemorrhagic areas visible through the peritoneum were common; they often extended into the muscle. Hemorrhagic areas at the base of the pectoral or pelvic fins and in the isthmus were frequent. Atrophy of the peduncle was noted in a few cases.

No significant organism could be found by direct examination nor could any be consistently isolated directly from these fish. A fluorescent pseudomonad was frequently recovered from the yearling bluebacks after they had become debilitated following injection of material from the small moribund fish. This was considered a secondary invader. The disease was infectious, as healthy yearling bluebacks were killed when injected with unfiltered and uncentrifuged saline extracts of the small moribund fish; and their appearance, while ill and at autopsy, resembled that of spontaneously infected fish. Seitz filtrates and heated material were innocuous.

KOKANEE BROOD OF 1949

The Winthrop station received 27,000, 2½-inch kokanee fingerlings, about 6 months old, from the Leavenworth station in October 1950. These fish had an insignificant loss at the Leavenworth station, but the mortality rate increased immediately upon arrival at Winthrop and the losses were high. The signs of the disease were similar to those of the bluebacks. All attempts to demonstrate an etiologic agent in tissue smears or by cultural methods failed. Nutritional deficiency was probably not of importance because similar fish at other stations on the same diet were not afflicted. Therapy by sulfonamides, antibiotics, and baths in disinfectants was of no benefit.

EXPERIMENTAL TRANSMISSION

Successful experimental transmission of the disease under natural conditions, using diseased fingerling bluebacks, was accomplished at the Winthrop station during the outbreak of March 1950. A screen partition was placed in a trough of diseased fish near the lower end of the trough, and about 4,000 diseased fish were held in the upper end. One hundred healthy chinook-salmon fingerlings (*O. tshawytscha*), 2 inches long and 4 months old, and 100 healthy blueback-salmon fingerlings, 1½ inches long and 3 months old, from disease-free stock at the Leavenworth station were placed in the lower compartment. During the next month 5 percent of the chinooks died from unknown causes. In the same period 95 percent of the bluebacks died showing evidence of the epizootic disease. They did not have the characteristic lesions of kidney disease and in our experience this bacterial infection has not been encountered in fish as small as those used in this experiment. Also, we have not transmitted kidney disease by contact or feeding experiments.

RELATION TO EPIZOOTIC AT LEAVENWORTH

The experiments and observations in transmitting the disease at Winthrop make it appear that a natural source of infection may be contaminated water. However, it is certain that during the 1950 epizootic at Winthrop, the water supply was not a continuing source of infection. Fish brought to the Winthrop station in March 1950 and cared for with equipment kept separate from the diseased fish did not contract the disease. Approximately 12,000 blueback fingerlings were shipped to Winthrop from Leavenworth on March 15, 1950, and an additional 50,000 on June 8. These fish averaged 1 and 1½ inches

long, respectively, and had hatched during December 1949 and January 1950, from eggs of the Winthrop brood of 1949 shipped to the Leavenworth station in November 1949. They arrived at the Winthrop station during the epizootic, and were held with insignificant losses until the next spring when they were planted. It appears that neither the general water supply nor the food was a source of infection and that careful sanitary measures were effective in limiting the spread of the disease.

It is believed that the disease at Winthrop, although complicated by the presence of bacterial pathogens, was similar to the disease at Leavenworth. This is evidenced by the lack of response to sulfonamide, antibiotic, or pyridylmercuric acetate therapy, and by the similar pathology. Hemorrhagic areas were observed on the fins and body surface, and evidence of inflammation was seen on the air bladder, visceral fat, and intestine. The intestine was often congested and filled with a yellowish fluid, the stomach with a whitish fluid.

DISCUSSION

The literature on virus diseases among fish is not extensive and deals mainly with lymphocystis. The lymphocystis virus disease of fish is characterized by fibroblasts which have undergone an enormous hypertrophy. References can be found in an article by Alexandrowicz (1951) and articles by Weissenberg (1951), some dating back to 1914.

Layman (1949) decribed a virus disease called red disease of carp. The disease was produced experimentally in healthy carp by rubbing an abraded area with a bacteria-free filtrate prepared from the skin of diseased carp. The disease had an incubation period of 3 days in warm water and of 3 weeks in cold water. Usually the first victims were the ripe fish, but 2½- to 3-month-old and 10- to 11-month-old fish were frequently attacked. The diseased fish were characterized by apathy, erection of scales, ascites, popeye, skin ulcers, and protrusion of the anal region. The intestine was inflamed, and the feces were dense, partially transparent, and stringy. The liver and gall bladder were enlarged. The liver was light green and dark in parts and finally became partially necrotic. The spleen was somewhat enlarged and very anemic in appearance. An acute form of this disease developed in early spring and abated by summer, while a chronic form progressed throughout the summer until the end of September.

A disease described by Pacheco and Guimaraes (1933) was attributed to a filtrable virus. The disease was called cryo-ichtyozoose because the virus acted on fish at low temperatures. The virulence diminished or even disappeared entirely at a temperature higher than 16° C. (60.8° F.). The disease was characterized by a high mortality, apathy, congestion at the base of the pectoral fins, pale gills and viscera—especially the liver; a mucous secretion in the stomach and mouth; and bile that was sticky and amber-colored.

Magarinos Torres and Pacheco (1934) later described this disease as stomatitis and demonstrated cytoplasmic inclusions in epithelial cells from the floor of the mouth where cloudy swelling was present. They were dealing with the *Acara*, a fish in an entirely different order from that containing the salmon.

No inclusion bodies that could be regarded as indicating virus infection were encountered in the blueback-salmon or kokanee fingerlings studied in the present investigation. Occasional red spherical cytoplasmic bodies were seen in the epithelial cells of the mouth and a few other areas. These were usually from 2 to 5 microns in diameter. Such bodies were found a little more commonly in diseased fish than in healthy fish of the same age. They resemble closely the bodies that have been previously described in a variety of human conditions and were thought by some to be associated with specific virus infections. However, they have been shown to be commonly present in the pharyngeal epithelium of normal humans (Macaulay 1951).

It appears possible that the disease described by the South American investigators is the same as that described in the present paper. It should be noted that their work involved fish of a different order from those we studied. Whether the two diseases are the same cannot be determined from present evidence. Findings in the present study suggested that only fish of the species *O. nerka* were susceptible. Infection did not occur in *O.*

tshawytscha, *O. kisutch*, or *Salmo clarki clarki* exposed under conditions that caused a high infection rate in *O. nerka*. We are inclinded to believe that the South American disease is not the same as the one described in this paper.

Schäperclaus (1941) described a virus disease of rainbow trout, in which the fish became dark in color. The posterior third of the kidney was swollen, the surface corrugated. The head kidney was arched in shape and mealy, pulpy, or pasty in consistency. The musculature over the kidney was softened. The liver was often light, yellowish, or spotty in appearance. The gall bladder was normal in half the cases investigated, otherwise it was small and filled with a yellowish liquid. The anal region was sometimes swollen and protruding. The disease was produced among healthy fish by injecting filtered material from suspensions of both diseased kidneys and livers. The investigator reported that the course of the disease is slow and that 30 to 60 percent of rainbow trout can be lost during the normal rearing period. He cited one example in which 80,000 rainbow-trout fingerlings, 5 to 7 centimeters long, died in 14 days as a result of this disease.

The disease among the blueback-salmon and kokanee fingerlings at the Winthrop and Leavenworth stations seems to differ enough from any diseases previously described to warrant its consideration as a previously undescribed disease caused by a filtrable agent.

The development of scoliosis and lordosis among the blueblack-salmon and kokanee fingerlings is of interest. Schräder (1930) mentioned that spinal-column deformities are more common in fish than in all other vertebrates. He described the anatomical changes of a number of curvatures in fish and reviewed the literature. Factors attributed as causes of curvatures are heredity; an unequal development of muscle or its elasticity; parasitic infections in which muscle or cartilage is destroyed; nutritional deficiences as avitaminosis or lack of phosphorus or calcium; and oxygen deficiency, unfavorable temperatures or improper water balance in the developing egg.

McCay and Tunison (1934) found that brook-trout fingerlings raised on a diet containing one-third sheep plucks preserved in 1-percent formalin developed scoliosis after about a year when they were approximately 6 inches long.

SUMMARY

1. An investigation was made of a disease affecting blueback-salmon and kokanee fingerlings (*Oncorhynchus nerka*) at the Leavenworth and Winthrop stations in 1951. The disease did not infect fingerlings of the chinook salmon (*O. tshawytscha*), silver salmon (*O. kisutch*), or cutthroat trout (*Salmo clarki clarki*).

2. The disease was characterized at the beginning of an outbreak by a great increase in its infectious nature, and a resulting increase in the mortality of the fish.

3. The total mortality in a population of 800,000 to 1,400,000 salmon ranged from 11 to 40 percent. A decrease in the epizootic appeared to follow sterilization of cleaning equipment. The incidence of the disease decreased during summer months, and it was suspected that the higher water temperatures were a favorable factor.

4. The disease was produced by material that passed through bacteria-proof filters. The infection that was produced in healthy salmon by injection of suspensions made from moribund fish resembled the disease as it occurred naturally during the epizootic. Some injected fish developed scoliosis or lordosis. Results of the injection of salmon with heated material were negative.

5. The larger fish in a group were attacked first, becoming infected when they were 1 to 1.5 inches in length and about 3 months old.

6. Diseased fish were lethargic and often exhibited hemorrhagic areas at the base of the fins and in the isthmus. The stomach was distended with a milky fluid; and the intestine appeared reddened and contained a watery, straw-colored fluid often tinged with blood. The bile appeared normal, but in greater quantity than usual. Hyperemic areas frequently were present on the air bladder, peritoneum, or fat.

7. Spinal deformities developed in later stages among some of the fish that survived the early acute stage of the epizootic.

8. Experimental infection was accomplished by placing healthy fish in one end of a trough that contained sick fish in the upper compartment.

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